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ORIGINAL RESEARCH

Coilin Affects the Prognosis of Hepatocellular Carcinoma Through Cell Cycle and Apoptosis

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Purpose: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality with a challenging prognosis. HCC lacks effective prognostic biomarkers. We investigated the diagnostic and prognostic value of COIL expression in HCC.

Patients and Methods: This study evaluated the expression and prognostic significance of COIL using data from the TCGA and local hospital samples, with 374 and 118 liver cancer patients in the TCGA database and local hospital, respectively. The techniques include bioinformatics analysis, qRT-PCR, immunohistochemistry (IHC), and in vitro cell experiments, which encompass CCK-8 assays, wound healing assays, and Transwell invasion assays. The relationship between COIL expression and clinical outcomes was assessed, and COIL's biological function in HCC was investigated through cellular assays.

Results: Analysis of cell lines and HCC tissue samples revealed that COIL mRNA or protein expression levels were significantly higher in HCC cell lines/tissues compared to normal liver cells/tissues. Univariate and multivariate analyses indicated that COIL is an independent prognostic factor for overall survival (OS) in HCC. Additionally, 14% of HCC patients had alterations in the COIL gene, and patients with COIL gene alterations had significantly lower OS ($p<0.001$) and disease-free survival (DFS) ($p<0.001$) compared to those without gene alterations. Knockdown of COIL expression inhibited the proliferation, migration, and invasion of Hep3B, HepG2, and Huh7. Compared to the control group, COIL knockdown cells showed a marked reduction in CDC25C and CCNB1 protein levels, suggesting that COIL knockdown leads to G2/M phase cell cycle arrest. After COIL knockdown, caspase-3 and BCL-2 protein levels were downregulated, while cleaved caspase and BAX protein levels were upregulated, indicating that COIL knockdown promotes apoptosis in liver cancer cells.

Conclusion: COIL is an independent predictor of prognosis. COIL's association with poor OS and its role in enhancing cancer cell proliferation and invasion highlight its potential as a therapeutic target.

Keywords: hepatocellular carcinoma, COIL, prognosis, overall survival

Introduction

Hepatocellular carcinoma (HCC) has the fifth highest cancer-related mortality rate and a poor prognosis.[1] In addition to chronic hepatitis virus infection, alcoholism and obesity are risk factors for hepatocellular carcinoma,[2] genetic factors also promote the occurrence of liver cancer.[3] Current treatment of HCC still relies mainly on hepatectomy. Although the multi-disciplinary development has made the treatment of HCC more perfect, it was still not satisfactory in terms of recurrence and prognosis.[4] Early diagnosis is an essential part of the treatment strategy. Serum alpha-fetoprotein (AFP) has been widely used in the diagnosis of HCC.[5] But it is not enough to screen all early-stage HCCs, especially those without elevated AFP.[6] Recent reports suggest that radiomics models have the potential to assess the Tumor Immune Microenvironment status within tumors and predict clinical outcomes as well as responses to immunotherapy. Despite the promising implications of these models, their clinical application remains limited.[7] Therefore, no biomarker with high specificity and sensitivity can predict the occurrence and prognosis of HCC. We hope to find biomarkers that are with high sensitivity to the diagnosis and prognosis.

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High-throughput sequencing has enabled scientists to identify new biomarkers associated with HCC prognosis. Coilin (COIL) expression was up-regulated in patients with HCC through bioinformatics. The protein p80-coilin, encoded by the COIL, is an integral component of Cajal bodies (CBs) (initially also called coiled bodies).[8] Studies found that p80-coilin was identified as a molecular marker of CBs.[9,10] CBs disruptions were associated with neurodegenerative diseases and potential cancers. Small nuclear ribonucleoproteins (snRNPs) are modified, assembled and recycled in CBs.[11,12] COIL's role in the biogenesis of snRNPs suggests that it could influence alternative splicing events in cancer cells. Dysregulation of splicing is a common feature in cancer, and alterations in coilin expression might lead to aberrant splicing patterns that contribute to HCC development. Both phosphorylation and methylation occurred on coilin.[13] Interestingly, accumulation of coilin was also found when some of the residues were mutated to non-phosphorylated alanine residues.[14,15] In addition, the human coilin interacting nuclear ATPase proteins are associated with P53 and NF-κB pathways to promote the development of tumors.[16] It has been confirmed that there were obvious coiled bodies in the nuclear plasma of different breast cancer cell lines.[17] Coilin has been shown to interact with proteins involved in cell cycle regulation. Changes in coilin expression could disrupt the normal control of cell cycle progression, potentially leading to uncontrolled cell division, a hallmark of cancer.[18] Animal studies showed that p80-coilin was delocalized from CBs in leukemia.[19] Existing studies have linked CBs to disease development rather than coilin itself. Therefore, the specific value of coilin in tumor development has been ignored.

However, its molecular mechanism in HCC is still unclear. Therefore, in this study, we utilized two databases, TCGA and our hospital data, to assess the impact of COIL on the prognosis of HCC patients. Through in vitro cell experiments, we provide evidence that COIL enhances cell proliferation, migration, and invasion. Consequently, COIL is anticipated to be both a promising biomarker and a potential therapeutic target for HCC patients.

Materials and Methods

Differential Expression of COIL

An analysis of COIL expression in all tumor types and normal tissues was conducted using the TIMER[20] database. Then, using the data of TCGA database, the COIL expression in 374 cases of HCC and 50 cases of normal liver tissues were compared, and the data were analyzed using the R language. We also analyzed the relative expression of COIL in patients from 900th hospital. Immunohistochemistry was performed in 118 cases of HCC from the 900th hospital. Forty-nine pairs of HCC and matched adjacent liver cancer tissues were obtained for qRT-PCR from patients undergoing HCC surgical resection at 900th hospital.

These 118 patients who underwent hepatectomy as the initial treatment at the 900th hospital from October 2016 to December 2019 were enrolled. The inclusion criteria are as follows: 1. HCC was pathologically diagnosed in all patients. 2. No other chemotherapy, radiotherapy and immunotherapy were performed before hepatectomy. 3. Patients were older than 18 years. The exclusion criteria were as follows: Individuals under 18 years of age who died from causes unrelated to cancer within one week after surgery, as well as those who underwent a second hepatectomy, were excluded from the study. Other baseline data were obtained from the hospital's case system. The last follow-up was on March 20, 2022. The expression of COIL was examined by IHC in 118 cases of HCC tissue.

Quantitative Real-Time PCR (qRT-PCR) of COIL

qRT–PCR was measured with the ABI 7900HT system with SYBR Select Master Mix (4472908, Thermo Fisher). The cycling parameters were as follows: 50°C for 2 minutes, 95°C for 2 minutes, then 95°C for 15s and 60°C for 1 minute, 40 cycles. The sequences of the primers are as follows: COIL forward, 5'-GAG GCG TTC TGG CTC AAA TG-3' and reverse, 5'-GGA AAC AGG ATG CCC TCG TC-3'; β-actin forward, 5'-TGA CGT GGA CAT CCG CAA AG-3' and reverse, 5'-CTG GAA GGT GGA CAG CGA GG-3'. The relative expression of COIL was calculated using the 2-ΔΔCq method.

Immunohistochemistry (IHC), Immunocytochemistry (ICC) and Western Blot

The 900th hospital provided all samples (Fujian, China). The sections were pre-treated using EDTA buffer for antigen retrieval. The anti-coilin antibody (1:200, ab87913, Abcam) was dropped onto sections, incubated at 4°C overnight. Secondary antibody (Kit-9901; Fuzhou Maixin Biotech) was dropped at room temperature for 30 minutes. The coloration

was achieved with diaminobenzidine (DAB). Negative control experiments were also performed where the sections were incubated with antibody diluent only, without the primary antibody. For Western blotting experiments, we used GAPDH antibody (diluted 1:5000) as a loading control to ensure equal protein loading across the gel. To further validate the accuracy of our results, each experiment included a blank control lane where no protein sample was loaded.

ICC was performed following standard protocols. L-02, HepG2, Huh7, or Hep3B cells were fixed in 4% Paraformaldehyde for 1 hour and permeabilized using 0.5% Triton X-100 (G1204, Servicebio) and then processed for incubation with antibodies listed above. The hepatocellular carcinoma cell lines HepG2, Hep3B, and Huh7 were purchased from Cyagen Biosciences (Guangzhou) Co., Ltd. The human normal liver cell line L02 was obtained from Beijing Baiao Bowei Biotechnology Co., Ltd.

Correlation Between COIL and Survival Outcome in TCGA and 900th Hospital

Cases with incomplete clinical data in the TCGA database were excluded. According to COIL median expression values, patients were grouped into low- and high-expression groups. The correlation between COIL and overall survival (OS) was analyzed. In addition, an analysis of gene alterations and their association with survival time was conducted. The relationship between the gene changes of COIL and prognosis was analyzed by cBioportal.[21] The selection condition of genomic profiles was set as "Enter a Z-Score threshold ±2.0".

We have grouped 118 patients from the 900th Hospital into two groups: the high COIL expression group and the low COIL expression group. Univariate or multivariate Cox analysis was applied to assess the risk factors of HCC. Multivariate analysis included factors with $p<0.05$.

Correlation Between COIL and Clinical Features

The UALCAN (https://ualcan.path.uab.edu/analysis.html) is an online database based on the TCGA, which can provide the different expressions of interest gene and the correlation with clinicopathological features.[22] The expression of COIL in different tumor grades, pathological stages, age, gender, race, and TP53 mutation status was analyzed by UALCAN, and box-plot visualization was performed.

Biological Function of COIL in Hepatocellular Carcinoma

To explore the biological function of COIL in liver cancer, in vitro cell functional experiments were conducted. The COIL shRNA lentiviral expression vector was used to transfect HepG2, Hep3B, and Huh7 cell lines, and stable COIL knockdown liver cancer cell lines were selected. COIL expression was validated using Western blotting and qRT-PCR. The proliferation, migration, and invasion abilities of the liver cancer cells were assessed using CCK-8 assays, wound healing assays, and Transwell invasion assays, respectively. Additionally, the expression levels of apoptosis and cell cycle-related proteins in liver cancer were examined.

Results

COIL Expression and HCC in TCGA

The expression of COIL in different tumor types in the TCGA database was analyzed. COIL expression was abnormal in 14 types of tumors, of which 10 were highly expressed (Figure 1A). Compared to normal liver tissue, HCC expresses significantly higher levels of COIL ($p<0.001$, Figure 1B). The higher expression of COIL in HCC was also found in 50 paired samples from TCGA-LIHC ($p<0.001$, Figure 1C).

We analyzed the correlation between COIL expression and survival prognosis in liver cancer patients and plotted survival curves. The Kaplan–Meier survival curve results showed that high COIL expression is significantly associated with poorer overall survival (OS) ($p<0.05$, Figure 2A). Basic clinical characteristics of 374 patients with HCC from TCGA database were summarized in Table 1. The results showed significant differences between the groups in terms of race, pathological stage, histological grade, and serum alpha-fetoprotein ($p<0.05$).

Furthermore, the results of Cox regression analysis were shown in Table 2. Univariate analyses revealed that many factors were associated with OS. COIL (HR 1.605, 95% CI: 1.133–2.274, p=0.008), tumor stage (HR 2.598, 95% CI:

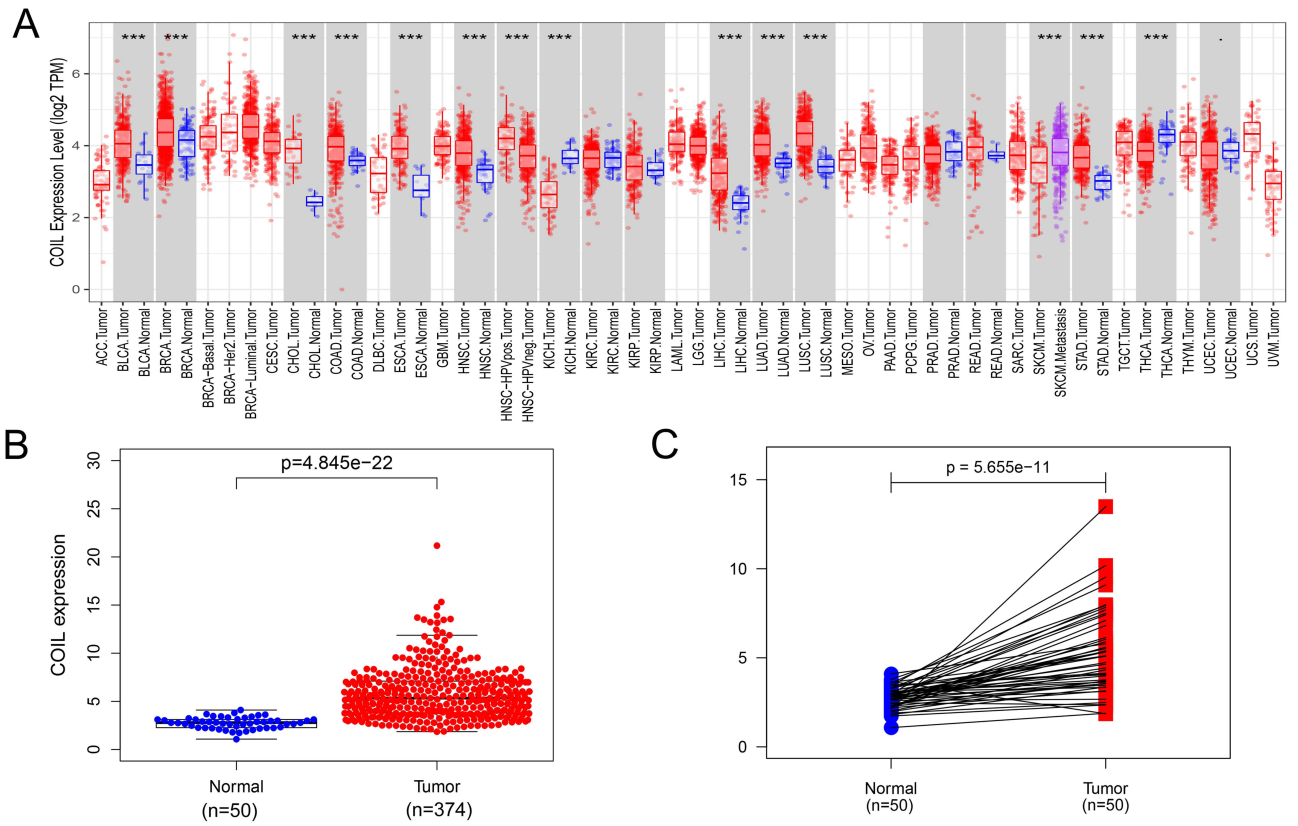

Figure 1 Expression of COIL in different tumors from TCGA. (**A**) COIL mRNA expression in different types of cancers. (**B**) COIL mRNA expression significantly upregulated in HCC compared with the non-HCC tissues in TCGA-LIHC. (**C**) Expression of COIL in paired samples of TCGA-LIHC. ***p value<0.001.

1.826–3.697, p<0.001), and metastasis stage (HR 4.077, 95% CI: 1.281–12.973, p=0.017) were related to OS. Multivariate analysis revealed that T staging (HR 2.661, 95% CI: 1.717–4.124, p<0.001) and COIL expression (HR 1.712, 95% CI: 1.093–2.683, p=0.019) were significantly associated with OS. These results indicated that COIL and tumor stage could be used as independent predictors of prognosis.

Correlation Between COIL Expression and Clinicopathological Features in TGCA Sample

The association between TCGA HCC samples and clinical phenotypes was analyzed using UALCAN online database. The patients were grouped based on different clinical characteristics, including race, gender, individual cancer stages, tumor grade, and nodal metastasis status, to analyze the relationship between COIL expression and these clinicopathological features. In HCC tissues of different races (Figure 2B), genders (Figure 2C), individual cancer stages (Figure 2D), tumor grades (Figure 2E), and nodal metastasis statuses (Figure 2F), COIL expression was consistently higher than in normal liver tissues. Notably, COIL expression levels increased with higher tumor grades (Figure 2E) and nodal metastasis statuses (Figure 2F).

COIL Gene Alterations and Survival Prognosis in TCGA

Next, we analyzed the relationship between COIL gene alterations and survival prognosis in HCC. As shown in Figure 3A, COIL gene alterations included amplifications, high mRNA expression, multiple alterations, and mutation. Among the 371 HCC patients, 51 (14%) exhibited gene alterations. Then, we further analyzed the survival prognosis of HCC with and without COIL gene alterations. The Kaplan–Meier survival curves showed that patients with COIL gene alterations had significantly poorer overall survival (OS) (p<0.001, Figure 3B) and disease-free survival (DFS) (p<0.001, Figure 3C) compared to those without COIL gene alterations. These results suggest that COIL gene alterations may also impact patient prognosis.

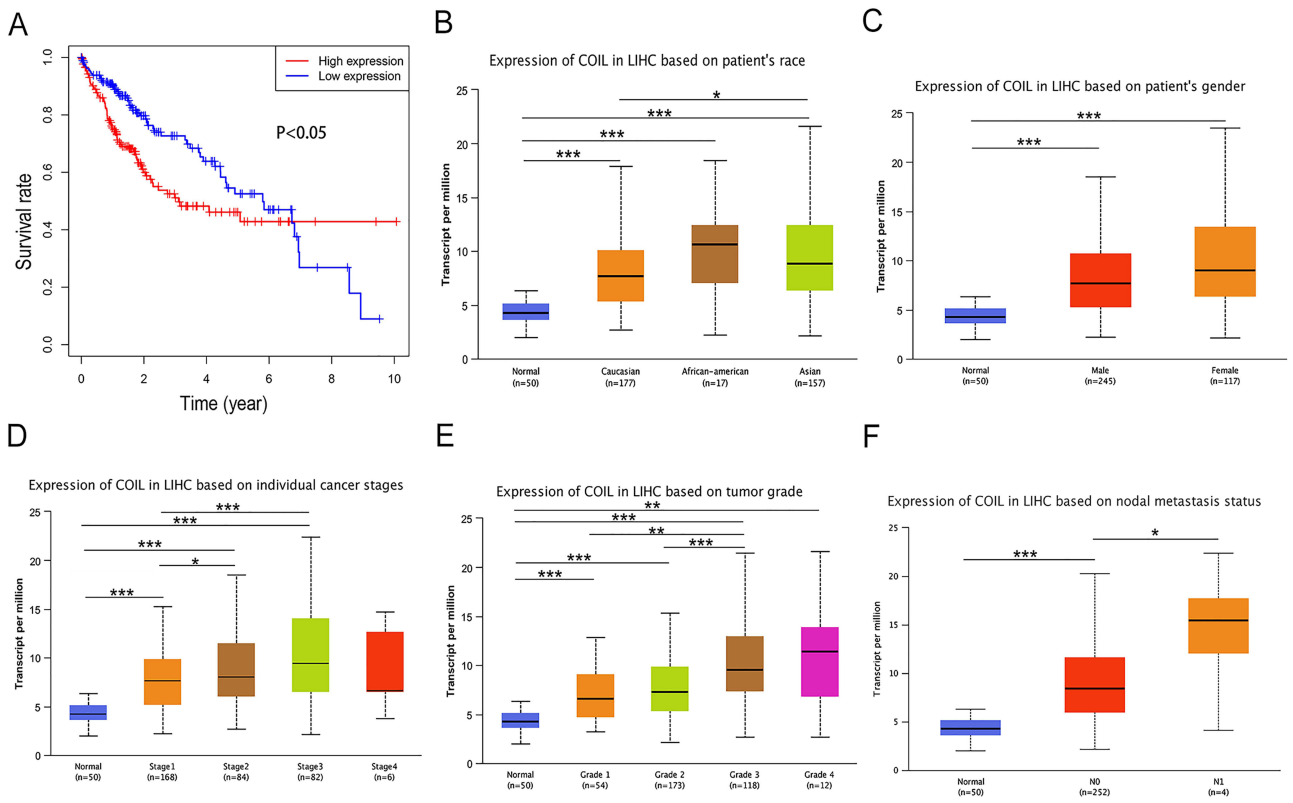

Figure 2 Correlation between COIL and survival prognosis or clinical characteristics in patients with HCC from TCGA. (**A**) High mRNA expression of COIL was correlated to poor overall survival. The expression of COIL in different race (**B**), gender (**C**), individual cancer stages (**D**), tumor grade (**E**), and nodal metastasis status (**F**). *p-value<0.05, **p value<0.01, ***p value<0.001.

COIL and AFP mRNA in TCGA

Using the mRNA expression data of COIL and AFP from patients with HCC in the TCGA database, we assessed their diagnostic value for HCC. ROC curves indicated that AFP had a moderate accuracy for predicting HCC (AUC = 0.720,

Table 1 Basic Clinical Features of Hepatocellular Carcinoma in 374 Cases from TCGA Database

Characteristic	Low Expression of COIL	High Expression of COIL	p Value
N	187	187	
T stage, n (%)			0.124
T1	102 (27.5%)	81 (21.8%)	
T2	43 (11.6%)	52 (14%)	
T3	33 (8.9%)	47 (12.7%)	
T4	6 (1.6%)	7 (1.9%)	
N stage, n (%)			0.623
N0	124 (48.1%)	130 (50.4%)	
N1	1 (0.4%)	3 (1.2%)	
M stage, n (%)			0.355
M0	128 (47.1%)	140 (51.5%)	
M1	3 (1.1%)	1 (0.4%)	
Gender, n (%)			0.077
Female	52 (13.9%)	69 (18.4%)	
Male	135 (36.1%)	118 (31.6%)	

(*Continued*)

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Table 1 (Continued).

Characteristic	Low Expression of COIL	High Expression of COIL	p Value
Race, n (%)			0.023
Asian	66 (18.2%)	94 (26%)	
Black or African American	7 (1.9%)	10 (2.8%)	
White	103 (28.5%)	82 (22.7%)	
Pathologic stage, n (%)			0.015
Stage I	98 (28%)	75 (21.4%)	
Stage II	42 (12%)	45 (12.9%)	
Stage III	32 (9.1%)	53 (15.1%)	
Stage IV	4 (1.1%)	1 (0.3%)	
Age, n (%)			0.277
<=60	83 (22.3%)	94 (25.2%)	
>60	104 (27.9%)	92 (24.7%)	
Histologic grade, n (%)			< 0.001
G1	38 (10.3%)	17 (4.6%)	
G2	102 (27.6%)	76 (20.6%)	
G3	42 (11.4%)	82 (22.2%)	
G4	3 (0.8%)	9 (2.4%)	
AFP (ng/mL), n (%)			< 0.001
<=400	128 (45.7%)	87 (31.1%)	
>400	14 (5%)	51 (18.2%)	
Child–Pugh grade, n (%)			0.363
A	119 (49.4%)	100 (41.5%)	
B	9 (3.7%)	12 (5%)	
C	1 (0.4%)	0 (0%)	
Vascular invasion, n (%)			0.491
No	112 (35.2%)	96 (30.2%)	
Yes	54 (17%)	56 (17.6%)	
Age, median (IQR)	62 (53, 69)	60 (51, 68)	0.142

Table 2 Univariate or Multivariate Cox Regression Analysis of Clinical Features and Overall Survival of Hepatocellular Carcinoma in TCGA

Characteristics	Total (N)	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	P Value	HR (95% CI)	P Value
T stage	370				
T1&T2	277	Reference			
T3&T4	93	2.598 (1.826–3.697)	**<0.001**	2.661 (1.717–4.124)	**<0.001**
N stage	258				
N0	254	Reference			
N1	4	2.029 (0.497–8.281)	0.324		
M stage	272				
M0	268	Reference			
M1	4	4.077 (1.281–12.973)	**0.017**	2.883 (0.850–9.777)	0.089
Gender	373				
Female	121	Reference			
Male	252	0.793 (0.557–1.130)	0.200		
Age	373				
<=60	177	Reference			
>60	196	1.205 (0.850–1.708)	0.295		

(*Continued*)

Table 2 (Continued).

Characteristics	Total (N)	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	P Value	HR (95% CI)	P Value
Race	361				
Asian	159	Reference			
White	185	1.323 (0.909–1.928)	0.144		
Black or African American	17	1.585 (0.675–3.725)	0.290		
AFP (ng/mL)	279				
<=400	215	Reference			
>400	64	1.075 (0.658–1.759)	0.772		
Child–Pugh grade	240				
A	218	Reference			
B	21	1.595 (0.757–3.361)	0.219		
C	1	2.138 (0.294–15.544)	0.453		
Vascular invasion	317				
No	208	Reference			
Yes	109	1.344 (0.887–2.035)	0.163		
COIL	373				
Low	187	Reference			
High	186	1.605 (1.133–2.274)	**0.008**	1.712 (1.093–2.683)	**0.019**

Note: Significant p-values are bolded.

95% CI: 0.668–0.773), while COIL showed higher accuracy (AUC = 0.909, 95% CI: 0.880–0.939) (Figure 3D). Based on the sensitivity and specificity of the ROC curves, the optimal cutoff values for COIL and AFP gene expression were determined. As shown in Supplementary Table 1, the positive predictive value (PPV) for COIL was 0.993, with a negative predictive value (NPV) of 0.350. For AFP, the PPV and NPV were 0.985 and 0.206, respectively.

We have also conducted a correlation study on AFP and COIL expression in liver cancer using the TIMER database. There is a strong positive correlation between AFP and COIL expression levels (cor=0.307, p<0.001).

Functional Analysis of COIL-Related Genes

The top 100 genes from cBioPortal and GEPIA were screened, and 41 of them were obtained by intersection (Figure 4A). These 41 COIL-related genes were assessed by KEGG and GO functional analysis. The results of KEGG analysis suggest that related genes may contribute to RNA transport regulation and spliceosome signal pathways (Figure 4B). GO enrichment analysis was also performed, including biological process (BP), cellular component (CC) and molecular function (MF). GO-CC analysis suggested that it was mainly enriched in the nucleus. For GO-BP, COIL-related genes were most enriched by covalent chromatin modification, oxidative phosphorylation, histone H4-K5, H4-K8, and H4-K16 acetylation. GO analysis results were visualized in Figure 4C. The PPI network was then performed in STRING database and visualized using Cytoscape software. Ten hub genes were screened according to MCC values. As shown in Figure 4D, the 10 hub genes are COIL, CBX1, MSH2, SUZ12, ATAD5, ZNF207, SUMO2, HNRNPU, SMARCE1, and KPNB1.

GSEA of COIL-Related Genes

GSEA was performed to found out potential signaling pathways. There were five signaling pathways significantly enriched at NOM P<0.05, including inhibitory and enhancing pathways. An overview of the GSEA results is shown in Figure 4E. Inhibitory signaling pathway was correlated with “glycosaminoglycan biosynthesis heparan sulfate”. And enhanced signaling pathways were correlated with “steroid hormone biosynthesis”, “ascorbate and aldarate metabolism”, “drug metabolism other enzymes”, and “metabolism of xenobiotics by cytochrome P450”.

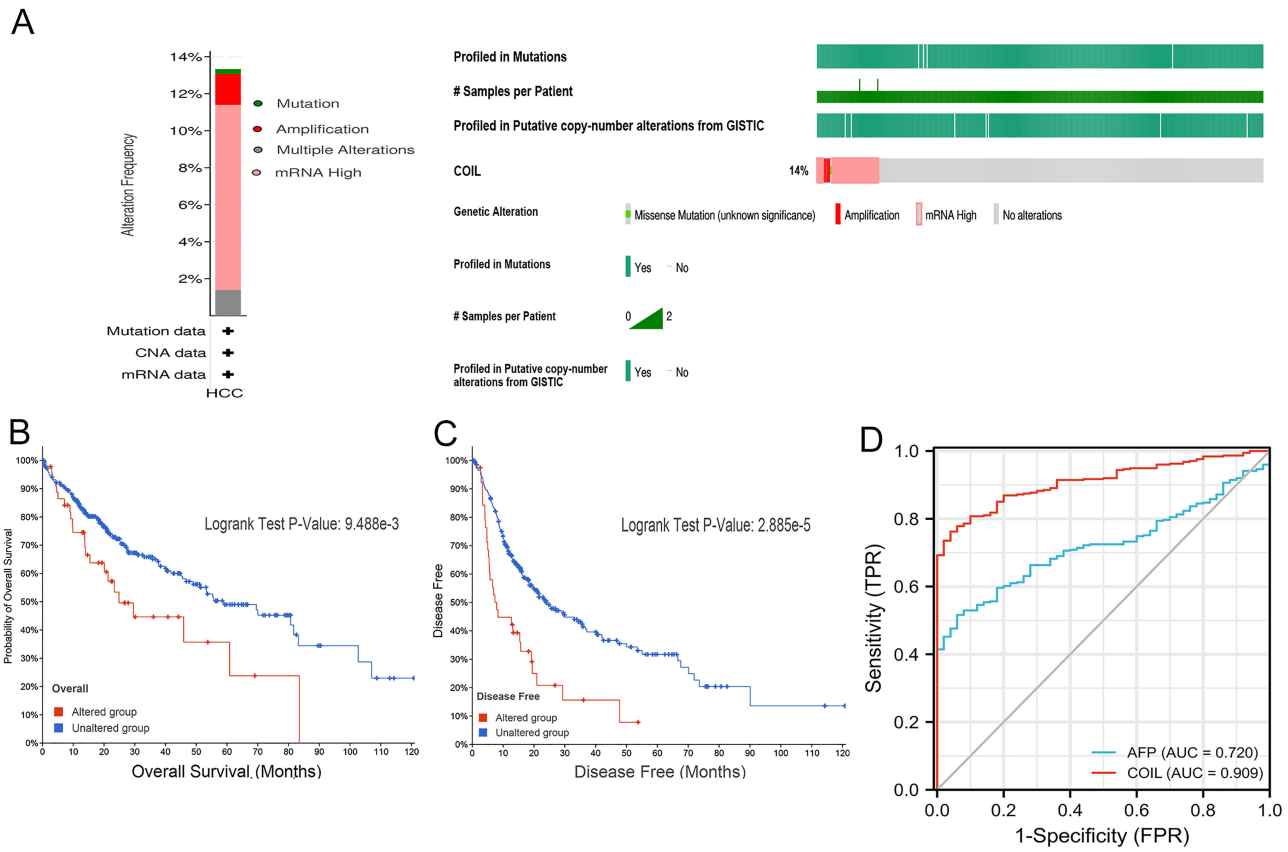

Figure 3 Relationship between COIL gene alterations and survival prognosis in patients with HCC from TCGA. (**A**) Gene alterations frequency and the mutations of COIL in HCC. The association between COIL gene alterations and overall survival (**B**) and disease-free survival (**C**) in HCC was evaluated by Kaplan–Meier survival curves. The AUC value of AFP is lower than that of COIL in the TCGA-LIHC (**D**).

Expression of COIL in Liver Cancer Cell Lines

COIL protein expression was measured in both normal liver cell lines and liver cancer cell lines. Immunohistochemical analysis of cell cytospins revealed that COIL protein levels were higher in the liver cancer cell lines Huh7, HepG2, and Hep3B compared to the normal liver cell line L02 (Figure 5A). In all three liver cancer cell lines, COIL protein was expressed in both the nucleus and the cytoplasm. Additionally, Western blot analysis assessed COIL protein levels across the four cell lines, showing that COIL protein expression was upregulated in liver cancer cell lines compared to the normal liver cell line L02 (Figure 5B).

COIL and Survival Outcome in Patients from the Hospital

Firstly, the COIL expression was examined by qRT-PCR in the paired HCC tissue and adjacent normal tissue of 49 patients. COIL expression was up-regulated in HCC tissues ($p<0.05$, Figure 5C). The results of Western blotting found that COIL protein expression was significantly higher in HCC tissues compared to normal liver tissues (Figure 5D).

Then this study collected 118 cases of HCC samples from the 900th hospital. Among the 118 HCC patients, 101 were male (85.6%) and 17 were female (14.4%). The ages ranged from 25 to 78 years, with a mean age of 52.24 years. Most patients were classified as Child–Pugh grade A (97.5%), and 78.8% were in stage I according to the TNM classification. Of the patients, 109 (92.4%) had no vascular invasion. The average OS was approximately 33.3 months. Immunohistochemistry was performed on the HCC tissues to analyze COIL protein expression. According to the semi-quantitative scoring system, the 118 patients were categorized into low-expression and high-expression groups. Our immunohistochemical results revealed that COIL was expressed in both the nucleus and the cytoplasm (Figure 5E). Kaplan–Meier survival analysis showed that high COIL expression was associated with poorer OS ($p<0.05$) (Figure 5F).

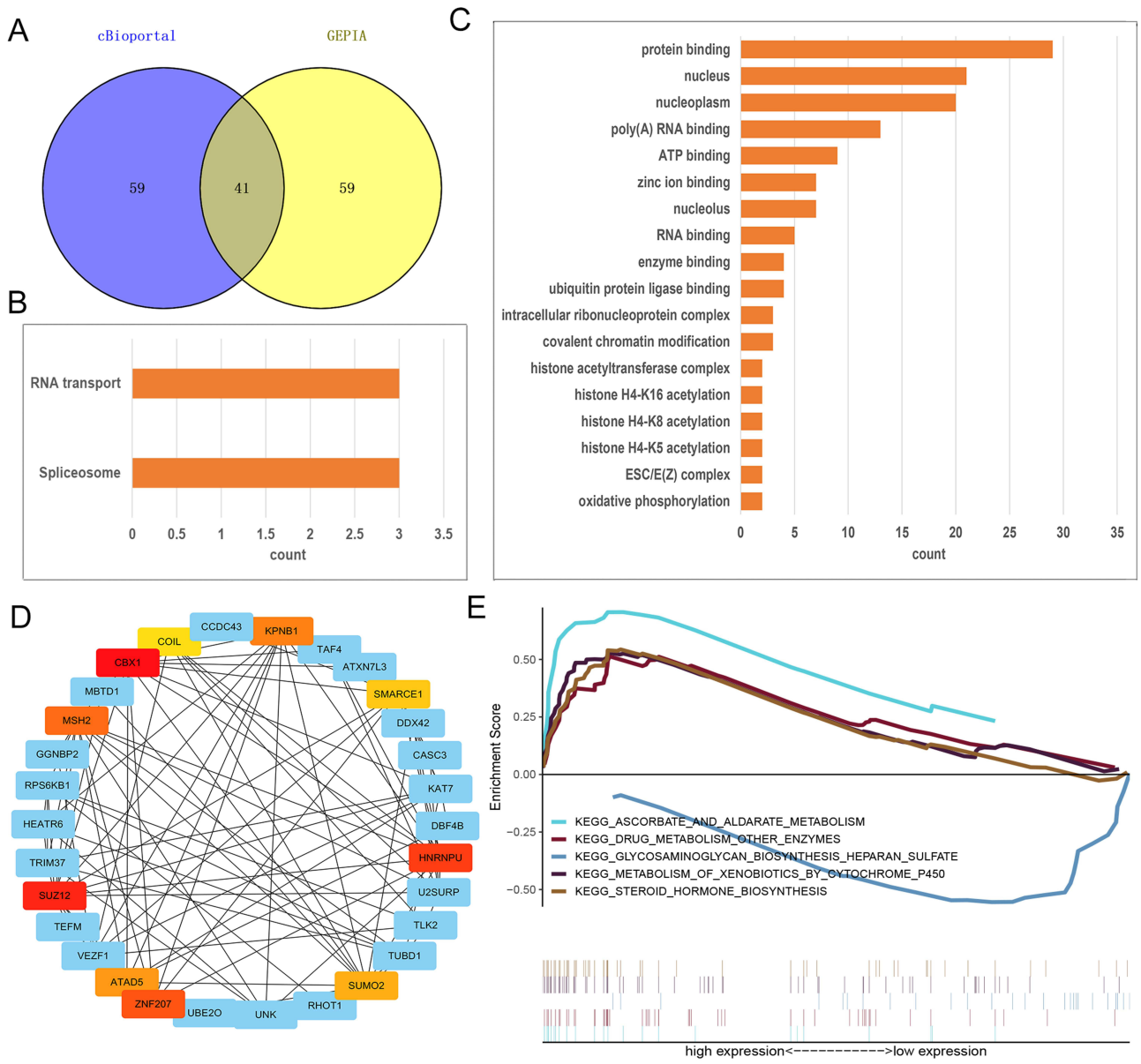

Figure 4 (**A**) Forty-one COIL-related genes were obtained through the intersection of cBioPortal and GEPIA databases. KEGG (**B**) or GO (**C**) analysis of these forty-one COIL-related genes was conducted. (**D**) A protein–protein interaction network was established and visualized. (**E**) GSEA of COIL-related genes.

In the univariate analysis, COIL expression (p<0.001, HR 8.377, 95% CI: 3.468–20.231), Glasgow Prognostic Score (GPS) (p<0.05, HR 3.614, 95% CI: 1.577–8.285), modified Glasgow Prognostic Score (mGPS) (p<0.05, HR 7.043, 95% CI: 2.982–16.637), age (p<0.05, HR 2.879, 95% CI: 1.262–6.567), tumor size (p<0.001, HR 4.204, 95% CI: 1.942–9.100), number of tumors (p<0.001, HR 8.304, 95% CI: 3.672–18.779), TNM stage (p<0.001, HR 23.482, 95% CI: 5.406–101.994), and BCLC stage (p<0.001, HR 0.143, 95% CI: 0.067–0.302) were significantly associated with overall survival (OS).

In the multivariate analysis, COIL expression (p<0.05, HR 3.568, 95% CI: 1.252–10.166), mGPS (p<0.05, HR 5.502, 95% CI: 1.143–26.477), tumor size (p<0.001, HR 8.415, 95% CI: 3.252–21.771), TNM stage (p<0.05, HR 15.046, 95% CI: 2.806–80.683), and BCLC staging (p<0.05, HR 0.210, 95% CI: 0.065–0.683) were associated with OS. COIL expression is one of the independent risk factors for OS (Table 3).

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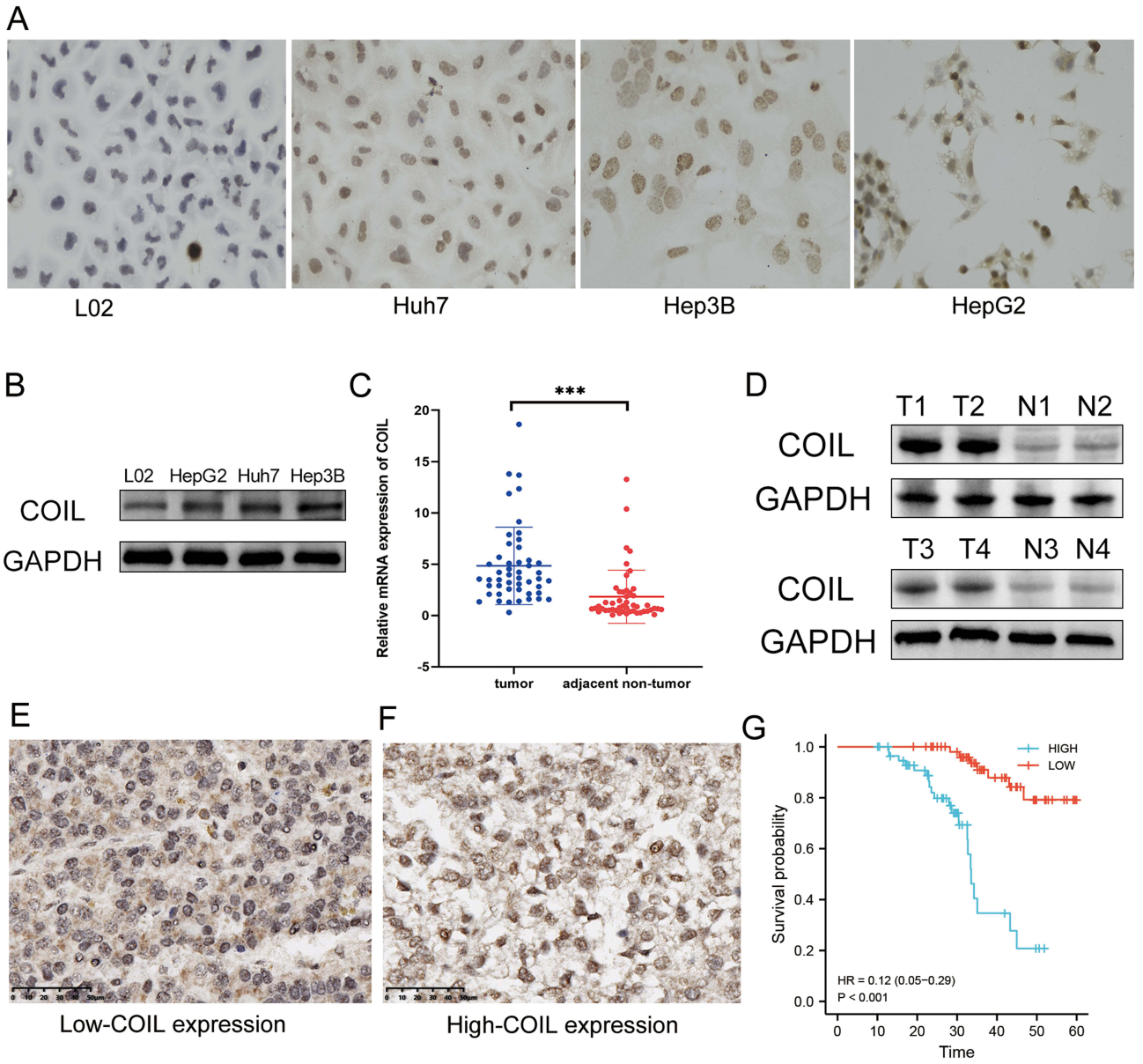

Figure 5 Expression of COIL in HCC cell lines and HCC tissue from hospital. The expression of COIL in liver cancer cell lines huh7, hepG2 and hep3B was higher than that in L02 by immunohistochemistry (**A**) and Western blotting (**B**). (**C**) The relative expression of COIL in HCC was significantly higher than that in normal liver tissue by qRT-PCR (***p value < 0.001). (**D**) The expression of COIL in HCC tissue was higher than that in normal liver tissue. (**E, F**) Representative immunohistochemistry results of low or high COIL expression. (**G**) High COIL expression was associated with poor overall survival.

COIL Promotes HCC Growth

To explore the biological role of COIL in HCC more deeply, we developed shRNA to knock down COIL in HepG2, Huh-7, and Hep3B cells. After selection with puromycin, we obtained stable liver cancer cell lines transfected with COIL-shRNA. To further validate the effect of COIL knockdown, we used qRT-PCR and Western blotting to analyze the transcriptional levels and protein expression of COIL in the cell lines (Figure 6A). The results of qRT-PCR and Western blotting revealed that, compared to the control group, COIL expression was significantly reduced. Therefore, the COIL-shRNA2-transfected cell lines were selected for further validation and analysis.

The transwell invasion assay revealed that significantly fewer COIL-knockdown cells invaded the lower chamber compared to the shNC controls (Figure 6B). The CCK-8 results indicated that COIL knockdown inhibited cell proliferation (Figure 6C). The results of scratch healing experiments show that COIL knockdown affects cell migration

Table 3 The Prognostic Value of Clinicopathologic Features on Overall Survival of Patients with Hepatocellular Carcinoma was Analyzed by Univariate and Multivariate Analysis

	N	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	*p* value	HR (95% CI)	*p* Value
COIL	118				
Low	59	Reference			
High	59	8.377 (3.468–20.231)	**<0.001**	3.568 (1.252–10.166)	**0.017**
SII					
<350	61	Reference			
≥350	57	0.771 (0.361–1.647)	0.501		
PNI					
≥45	109	Reference			
<45	9	1.959 (0.454–8.447)	0.367		
PLR					
<150	97	Reference			
≥150	21	1.418 (0.536–3.750)	0.482		
NLR					
≤3	99	Reference			
>3	19	1.869 (0.793–4.402)	0.153		
GPS					
0	81	Reference			
1+2	37	3.614 (1.577–8.285)	**0.002**	0.851 (0.166–4.360)	0.847
mGPS					
0	96	Reference			
1+2	22	7.043 (2.982–16.637)	**<0.001**	5.502 (1.143–26.477)	**0.033**
Gender					
Male	101	Reference			
Female	17	0.606 (0.183–2.009)	0.413		
Age (years)					
≤65	100	Reference			
>65	18	2.879 (1.262–6.567)	**0.012**	2.541 (0.918–7.037)	0.073
Child–Pugh grade					
Child–Pugh A	115	Reference			
Child–Pugh B	3	3.429 (0.797–14.762)	0.098		
Serum APF (ng/mL)					
≤400	85	Reference			
>400	33	1.595 (0.735–3.460)	0.237		
Cirrhosis					
Yes	66	Reference			
No	52	0.798 (0.358–1.777)	0.581		
Tumor size (cm)					
≤5	75	Reference			
>5	43	4.204 (1.942–9.100)	**<0.001**	8.415 (3.252–21.771)	**<0.001**
Number of tumors					
1	104	Reference			
≥2	14	8.304 (3.672–18.779)	**<0.001**	1.214 (0.360–4.093)	0.754
TNM stage					
Stage I+II	111	Reference			
Stage III+IV	7	23.482 (5.406–101.994)	**<0.001**	15.046 (2.806–80.683)	**0.002**
BCLC stage					
B+C	23	Reference			
0+A	95	0.143 (0.067–0.302)	**<0.001**	0.210 (0.065–0.683)	**0.010**

Note: Significant p-values are bolded.

Abbreviations: SII, Systemic Immune-Inflammation Index; PNI, Prognostic Nutritional Index; PLR, Platelet-to-Lymphocyte Ratio; NLR, Neutrophil-to-Lymphocyte Ratio; GPS, Glasgow Prognostic Score; mGPS, Modified Glasgow Prognostic Score; BCLC, Barcelona Clinic Liver Cancer Staging.

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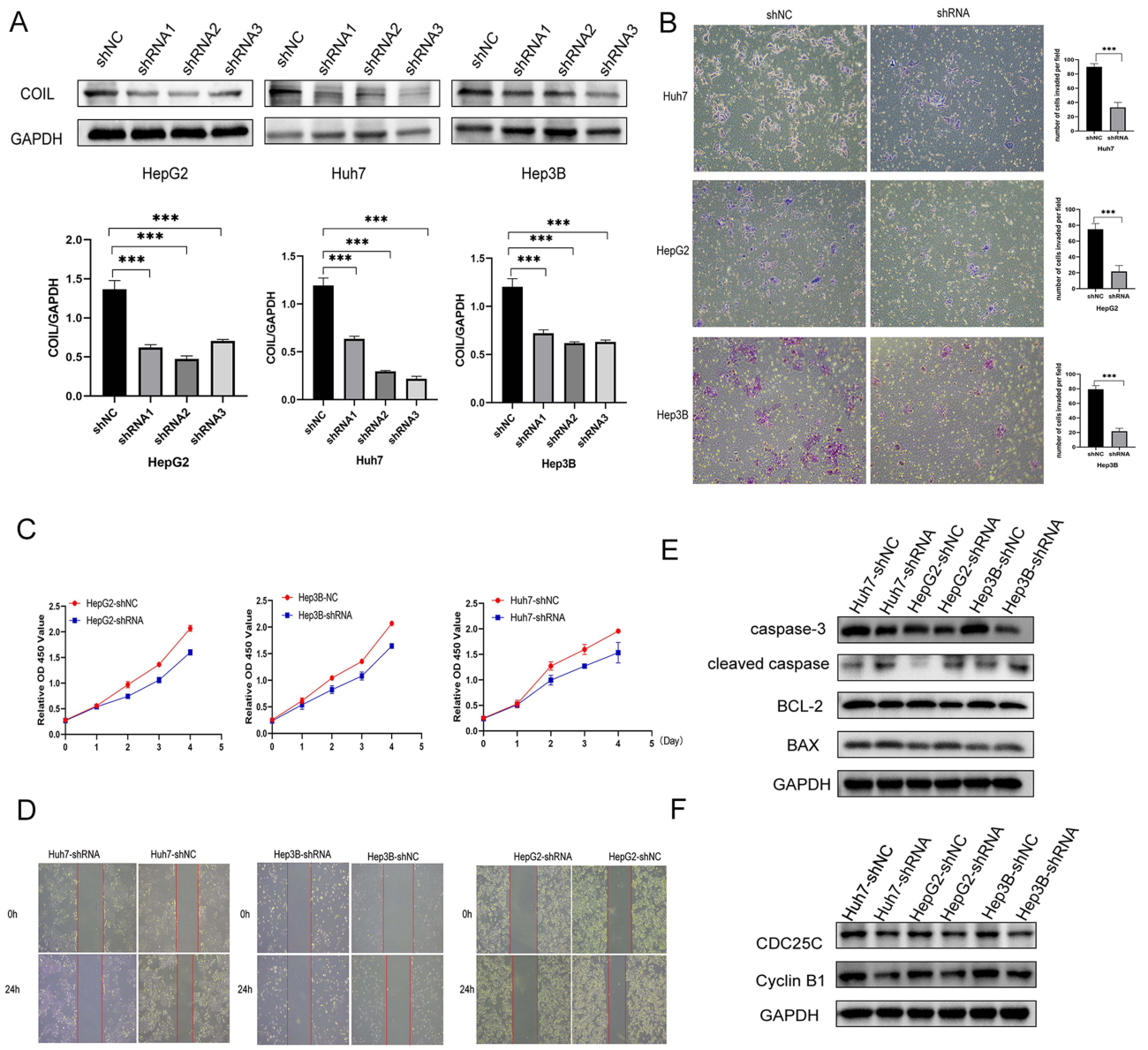

Figure 6 (**A**) Knockdown of COIL in HepG2, Huh7, and Hep3B (***p value < 0.001). (**B**) The effect of COIL on cell invasion ability was determined by Transwell invasion assay. (**C**) The effect of COIL on cell proliferation was determined by CCK-8 assay. (**D**) The cell migration was evaluated by scratch wound healing dynamic test. Western blotting detected the expression of apoptosis (**E**) and cell cycle-related genes (**F**).

(Figure 6D). COIL knockdown resulted in a decrease in the levels of caspase-3 and BCL-2 proteins, while the levels of cleaved caspase and BAX proteins were increased, indicating that COIL knockdown promotes cell apoptosis (Figure 6E). COIL knockdown resulted in a significant reduction in the expression levels of CDC25C and CCNB1 proteins (Figure 6F). This suggests that COIL knockdown leads to cell cycle arrest at the G2/M phase.

Analysis of Drug Sensitivity

We analyzed the drug sensitivity data retrieved from the GDSC database to investigate the potential function of COIL in predicting therapeutic response. We assessed the correlation between COIL expression levels and drug sensitivity. The results indicate that increased sensitivity to sorafenib, nilotinib, methotrexate, and gemcitabine is linked to higher COIL expression. In contrast, COIL expression was found to be associated with reduced sensitivity to Bryostatin-1 and lapatinib (Figure 7). These observations imply that COIL expression may influence the responsiveness to specific anti-cancer medications.

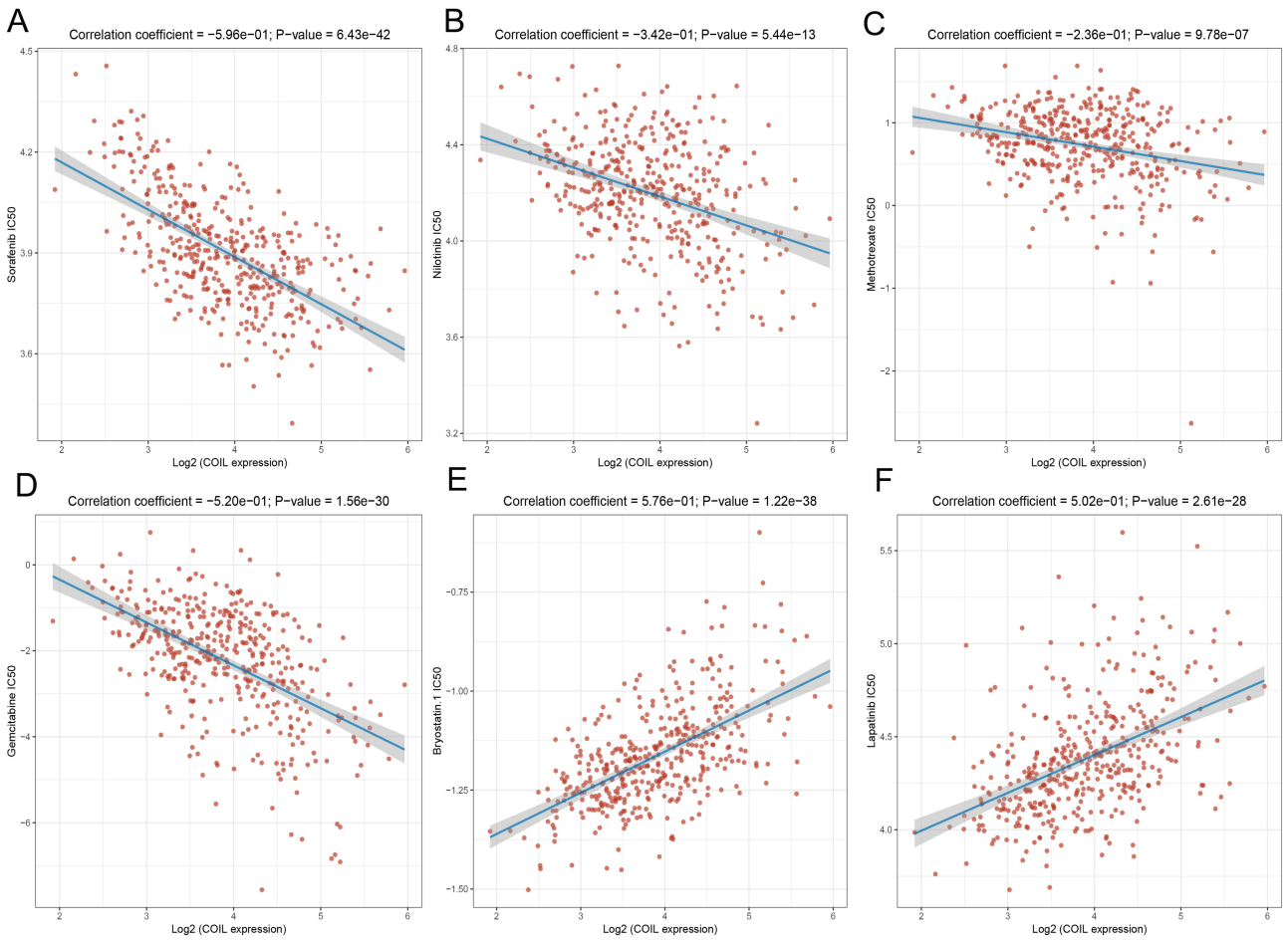

Figure 7 Correlation analysis between COIL expression and antitumor drug sensitivity. The results indicate that increased sensitivity to sorafenib (**A**), nilotinib (**B**), methotrexate(**C**), and gemcitabine (**D**) is linked to higher COIL expression. In contrast, COIL expression was found to be associated with reduced sensitivity to Bryostatin-1 (**E**) and lapatinib (**F**).

Discussion

Currently, AFP, as the most frequently used biological marker, is still unable to independently predict the prognosis of HCC. Not all HCC is accompanied by the increase of AFP. Moreover, studies have shown that AFP cannot predict the survival and prognosis of patients.[6] Therefore, it needs to study the prognostic biomarkers and their possible molecular mechanisms of HCC. In this work, we tried to reveal the relationship between COIL and the prognosis of HCC. We determined the relationship between COIL and prognosis of HCC by bioinformatics, IHC, WB, and qRT-PCR. The data of TCGA and 900th hospital showed that the COIL expression in HCC was significantly higher than that in non-HCC, both in terms of overall samples and paired samples. Moreover, the higher expression of COIL, the shorter OS of HCC. Multivariate analysis showed that COIL was an independent prognostic factor of HCC. Our study found that the mutation rate of COIL gene was 14% in patients with HCC. COIL gene changes were associated with OS and DFS. These results demonstrated that gene changes of COIL were closely related to the prognosis of HCC. COIL may be a risk factor in certain types of cancer.[18] We also verified the diagnosis value of COIL in HCC. The results show that COIL has a higher AUC value than AFP. According to our study, we believe that COIL is a potential biomarker. Therefore, COIL deserves further clinical verification as a potential prognostic marker.

Our study has identified alterations in the COIL gene in hepatocellular carcinoma (HCC), encompassing gene amplifications, high mRNA expression, multiple alterations, and mutations. These alterations may be associated with a variety of molecular mechanisms, including specific mutations and epigenetic changes. Specifically, the amplifications and high expression of the COIL gene likely reflect an increase in gene copy number, which could lead to overexpression of the COIL protein, thereby affecting the biological behavior of hepatocarcinoma cells. Regarding epigenetic changes, while our

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study did not directly investigate these, previous research suggests that epigenetic regulation, such as DNA methylation, may also play a role in the regulation of COIL gene expression.

Much remains unknown about COIL's role in tumorigenesis and pathogenesis, although sufficient studies have found links between CBs and specific human diseases. COIL is an important part and marker of CBs, and the most extensive function of CBs is the assembly of RNP.[13] Numerous studies on COIL have been conducted, the majority of which focus on its association with Cajal bodies (CBs), with fewer investigations examining COIL independent of CBs. Telomere shortening is the basis of cellular aging, but cancer avoids aging by lengthening telomeres by activating the ribonucleoprotein telomerase.[23] Reports that Coil-SiRNA eliminates telomerase accumulation on telomeres, as opposed to control-siRNA, and that residual CBs lacking COIL may not be able to participate in telomerase transport confirm the need for COIL for endogenous telomerase recruitment.[24] Chromosomal instability can be observed in most cancers, and the marked reduction of centromeric proteins in cells with chromosomal instability may be due to abnormal centromeric localization of COIL.[25]

A H/ACA motif in telomerase RNA is present in small Cajal body RNAs, which is in CBs.[26] In fact, telomerase RNAs mature in CBs, suggesting that CBs play an important role in maintaining telomere length.[27] Since telomere maintenance defects have been found in various cancers, coilin may be influencing tumors through telomeres. In addition, proliferative signals may be transmitted to the nucleus by CBs.[28] In COIL knockout cells, CBs did not store fibrillarin, and could not recruit U3 small nucleolar RNA.[11] Song et al found that down-regulation of coilin can inhibit cell growth and decrease cell survival rate, and the decrease of CBs formation rate is related to the enhancement of SAB-Gal staining. Coilin plays an important role in cisplatin-induced premature senescence.[29] Furthermore, it was found that the depletion of coilin did lead to the disintegration of CBs.[30]

While our in vitro studies have provided valuable insights into the potential role of COIL in hepatocellular carcinoma, it is imperative to underscore the significance of in vivo experiments. In vivo models offer a more comprehensive understanding of the tumor microenvironment and the systemic effects of COIL on tumor growth and progression. The translation of our findings to an animal model is crucial for validating the functional relevance of COIL in the pathogenesis of liver cancer. Therefore, we strongly advocate for the necessity of conducting in vivo studies to corroborate our in vitro observations. These experiments will not only confirm the biological significance of COIL but also pave the way for future therapeutic interventions targeting this molecule in hepatocellular carcinoma. There is still a need to explore the specific mechanism of action and regulatory pathways of COIL. This is also the direction we need to tackle in the future.

In this study, COIL promoted the proliferation, migration, and invasion of HCC. COIL knockdown promoted apoptosis and led to G2/M phase cell cycle arrest. It has been previously reported that COIL overexpression increases the percentage of the S and G2/M phases of the cell cycle and affects the proliferation rate,[31] which is consistent with our findings. In addition, single nucleotide polymorphisms of COIL can also lead to changes in cell growth and cell cycle.[18]

Conclusion

In conclusion, up-regulated expression of COIL is associated with poor OS in patients with HCC. Knocking down COIL expression not only inhibited the proliferation, migration, and invasion of hepatocellular carcinoma cells but also promoted apoptosis and cell cycle arrest.

Data Sharing Statement

The data that support the findings of this study are available in TCGA at https://portal.gdc.cancer.gov/. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

All the research data of TCGA are from open online databases, and all written informed consent can be guaranteed.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *Ca A Cancer J Clin*. 2021;71(1):7–33. doi:10.3322/caac.21654
2. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*. 2007;132(7):2557–2576. doi:10.1053/j.gastro.2007.04.061
3. Ally A, Balasundaram M, Carlsen R. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell*. 2017;169 (7):1327–1341.e1323. doi:10.1016/j.cell.2017.05.046
4. Finn RS, Zhu AX, Farah W, et al. Therapies for advanced stage hepatocellular carcinoma with macrovascular invasion or metastatic disease: a systematic review and meta-analysis. *Hepatology*. 2018;67(1):422–435. doi:10.1002/hep.29486
5. Wu M, Liu H, Liu Z, Liu C, Zhang A, Li N. Analysis of serum alpha-fetoprotein (AFP) and AFP-L3 levels by protein microarray. *J Int Med Res*. 2018;46(10):4297–4305. doi:10.1177/0300060518789304
6. Park SJ, Jang JY, Jeong SW, et al. Usefulness of AFP, AFP-L3, and PIVKA-II, and their combinations in diagnosing hepatocellular carcinoma. *Medicine*. 2017;96(11):e5811. doi:10.1097/MD.0000000000005811
7. Wu J, Liu W, Qiu X, et al. A noninvasive approach to evaluate tumor immune microenvironment and predict outcomes in hepatocellular carcinoma. *Phenomics*. 2023;3(6):549–564. doi:10.1007/s43657-023-00136-8
8. Machyna M, Neugebauer KM, Staněk D. Coilin: the first 25 years. *RNA Biol*. 2015;12(6):590–596. doi:10.1080/15476286.2015.1034923
9. Cioce M, Lamond AI. Cajal bodies: a long history of discovery. *Annu Rev Cell Dev Biol* 2005;21:105–131. doi:10.1146/annurev.cellbio.20.010403.103738
10. Raska I, Andrade LE, Ochs RL, et al. Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. *Exp Cell Res*. 1991;195(1):27–37. doi:10.1016/0014-4827(91)90496-H
11. Jády BE, Darzacq X, Tucker KE, Matera AG, Bertrand E, Kiss T. Modification of Sm small nuclear RNAs occurs in the nucleoplasmic Cajal body following import from the cytoplasm. *EMBO J*. 2003;22(8):1878–1888. doi:10.1093/emboj/cdg187
12. Nesic D, Tanackovic G, Krämer A. A role for Cajal bodies in the final steps of U2 snRNP biogenesis. *J Cell Sci*. 2004;117(Pt 19):4423–4433. doi:10.1242/jcs.01308
13. Trinkle-Mulcahy L, Sleeman JE. The Cajal body and the nucleolus: ”In a relationship” or ”It’s complicated”? *RNA Biol*. 2017;14(6):739–751. doi:10.1080/15476286.2016.1236169
14. Hebert MD, Matera AG. Self-association of coilin reveals a common theme in nuclear body localization. *Mol Biol Cell*. 2000;11(12):4159–4171. doi:10.1091/mbc.11.12.4159
15. Hearst SM, Gilder AS, Negi SS, et al. Cajal-body formation correlates with differential coilin phosphorylation in primary and transformed cell lines. *J Cell Sci*. 2009;122(Pt 11):1872–1881. doi:10.1242/jcs.044040
16. Yan Y, Yuan X, Xue C, He Y. Human coilin interacting nuclear ATPase protein in cancer: uncovering new insights into pathogenesis and therapy. *Am J Transl Res*. 2020;12(7):4051–4058.
17. Ochs RL, Stein TW Jr, Tan EM, Keck WM. Coiled bodies in the nucleolus of breast cancer cells. *J Cell Sci*. 1994;107(Pt 2):385–399. doi:10.1242/jcs.107.2.385
18. Yao Y, Tan HW, Liang ZL, Wu GQ, Xu YM, Lau ATY. The impact of coilin nonsynonymous SNP variants E121K and V145I on cell growth and Cajal body formation: the first characterization. *Genes*. 2020;11(8):895. doi:10.3390/genes11080895
19. Polak PE, Simone F, Kaberlein JJ, Luo RT, Thirman MJ. ELL and EAF1 are Cajal body components that are disrupted in MLL-ELL leukemia. *Mol Biol Cell*. 2003;14(4):1517–1528. doi:10.1091/mbc.e02-07-0394
20. Li T, Fan J, Wang B, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res*. 2017;77(21):e108–e110. doi:10.1158/0008-5472.CAN-17-0307
21. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signaling*. 2013;6(269):pl1. doi:10.1126/scisignal.2004088
22. Chandrashekar DS, Bashel B, Balasubramanya SAH, et al. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. *Neoplasia*. 2017;19(8):649–658. doi:10.1016/j.neo.2017.05.002
23. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur. J. Cancer*. 1997;33(5):787–791. doi:10.1016/S0959-8049(97)00062-2
24. Stern JL, Zyner KG, Pickett HA, Cohen SB, Bryan TM. Telomerase recruitment requires both TCAB1 and Cajal bodies independently. *Mol Cell Biol*. 2012;32(13):2384–2395. doi:10.1128/MCB.00379-12
25. Kazami T, Nie H, Satoh M, et al. Nuclear accumulation of annexin A2 contributes to chromosomal instability by coilin-mediated centromere damage. *Oncogene*. 2015;34(32):4177–4189. doi:10.1038/onc.2014.345
26. Kiss T, Fayet-Lebaron E, Jády BE. Box H/ACA small ribonucleoproteins. *Mol Cell*. 2010;37(5):597–606. doi:10.1016/j.molcel.2010.01.032
27. Venteicher AS, Abreu EB, Meng Z, et al. A human telomerase holoenzyme protein required for Cajal body localization and telomere synthesis. *Science*. 2009;323(5914):644–648. doi:10.1126/science.1165357
28. Claus P, Doring F, Gringel S, et al. Differential intranuclear localization of fibroblast growth factor-2 isoforms and specific interaction with the survival of motoneuron protein. *J Biol Chem*. 2003;278(1):479–485. doi:10.1074/jbc.M206056200
29. Song Y, Niu J, Yue Z, Gao R, Zhang C, Ding W. Increased chemo-sensitivity by knockdown coilin expression involved acceleration of premature cellular senescence in HeLa cells. *Biochem Biophys Res Commun* 2017;489(2):123–129. doi:10.1016/j.bbrc.2017.05.108
30. Strzelecka M, Oates AC, Neugebauer KM. Dynamic control of Cajal body number during zebrafish embryogenesis. *Nucleus*. 2010;1(1):96–108. doi:10.4161/nucl.1.1.10680
31. Velma V, Carrero ZI, Allen CB, Hebert MD. Coilin levels modulate cell cycle progression and γH2AX levels in etoposide treated U2OS cells. *FEBS Lett*. 2012;586(19):3404–3409. doi:10.1016/j.febslet.2012.07.054